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Decision tree analysis of clinical data to aid diagnostic reasoning for equine laminitis: a cross-sectional study.

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24 **Abstract**

25 The objective of this cross-sectional study was to compare the prevalence of selected clinical signs in
26 laminitis cases and non-laminitic but lame controls to evaluate their capability to discriminate laminitis from
27 other causes of lameness. Participating veterinary practitioners completed a checklist of laminitis-associated
28 clinical signs identified by literature review. Cases were defined as horses/ponies with veterinary-diagnosed,
29 clinically apparent laminitis; controls were horses/ponies with any lameness other than laminitis.
30 Associations were tested by logistic regression with adjusted odds ratios (OR) and 95% confidence intervals,
31 with veterinary practice as an *a priori* fixed effect. Multivariable analysis using graphical classification tree-
32 based statistical models linked laminitis prevalence with specific combinations of clinical signs. Data were
33 collected for 588 cases and 201 controls. Five clinical signs had a difference in prevalence of greater than
34 +50%: 'reluctance to walk' (OR 4.4, 'short, stilted gait at walk' (OR 9.4), 'difficulty turning' (OR 16.9),
35 'shifting weight' (OR 17.7) and 'increased digital pulse' (OR 13.2) (all $P<0.001$). 'Bilateral forelimb
36 lameness' was the best discriminator; 92% of animals with this clinical sign had laminitis (OR 40.5,
37 $P<0.001$). If, in addition, horses/ponies had an 'increased digital pulse', 99% were identified as laminitis.
38 'Presence of a flat/convex sole' also significantly enhanced clinical diagnosis discrimination (OR 15.5,
39 $P<0.001$). This is the first epidemiological laminitis study to use decision-tree analysis, providing the first
40 evidence-base for evaluating clinical signs to differentially diagnose laminitis from other causes of lameness.
41 Improved evaluation of the clinical signs displayed by laminitic animals examined by first-opinion
42 practitioners will lead to equine welfare improvements.

43

44

45

46 **Introduction**

47 Equine laminitis is a painful disease of the foot that affects equidae worldwide (Mellor and others 2001;
48 Wylie and others 2011). The insidious nature of the disease and potential for unrelenting pain often
49 necessitates euthanasia of the affected animal on welfare grounds (Hunt 1993; Menzies-Gow and others
50 2010b). Effective diagnosis is necessary to allow prompt instigation of palliative and therapeutic treatments,
51 to maximise recovery prospects.

52 In equine medicine, 'laminitis' is used to describe animals presenting with pain localised to the lamellar
53 region of the foot, with or without concurrent solar pain under the distal margin of the distal phalanx (Stashak
54 2002). There are no universally accepted gold-standard techniques for the detection and quantification of the
55 four stages of laminitis (Eustace 2010; Herthel and Hood 1999; Hunt and Wharton 2010; Menzies-Gow and
56 others 2010c; Swanson 1999). Acute laminitis arises with the development of clinical signs appreciable as
57 changes in the normal stance and gait of the animal (Baxter 1994; Coffman and Garner 1972; Swanson 1999).
58 Acute laminitis either progresses to the subacute form or to the chronic form of the disease. The subacute
59 stage can either persist, develop to chronic laminitis, or lead to complete recovery. Development of chronic
60 laminitis usually results in a cycle of recurrent episodes (Hood 1999). The terminology used to describe
61 chronic laminitis is extremely variable (Parks and Mair 2009), but is often taken to describe progression from
62 acute laminitis to failure of the SADP resulting in dislocation of the DP following detachment of the hoof
63 wall (Grosenbaugh and others 1999).

64 Laminitis is necessarily commonly diagnosed solely on the presence of a combination of characteristic
65 clinical signs (Baxter 1994; Vinuela-Fernandez *et al.* 2011a). Diagnostic challenges are compounded by the
66 multifactorial aetiology of the disease, which can arise as a consequence of systemic inflammatory disease,
67 endocrine disease or abnormal weight/load bearing which may initiate distinct pathophysiological processes
68 as reviewed by Eades (2010). However, the common feature of all cases of laminitis is the induction of
69 pathological changes within the SADP, resulting in overt foot pain and clinical signs related to lameness
70 (Baxter 1994; Budras and others 2009a; Budras and others 2009b).

71 Despite the perceived importance there is remarkably little evidence-based data regarding the clinical
72 presentation of laminitis (Eustace 2010; Hunt and Wharton 2010; Mellor and others 2001; Wylie and others
73 2013a), adding to inherent difficulties in establishing accurate diagnosis of laminitis due to the non-specific
74 nature of clinical signs and the absence of robust case definitions. Furthermore, there is no general agreement
75 regarding standardised criteria to diagnose laminitis or to classify affected animals based on the phase of
76 disease progression and/or disease aetiology (Parks and Mair 2009; Rohrbach and others 1995). The
77 debilitating consequences of laminitis do, however, require prompt veterinary intervention and accurate
78 diagnosis is therefore essential.

79 All the factors outlined above complicate the overall challenge of diagnostic reasoning based on clinical
80 signs, presenting the veterinary clinician with a challenge to diagnose laminitis differentially from other
81 forms of orthopaedic disorder. Therefore, the aim of this study was to compare the prevalence of selected
82 clinical signs in laminitis and non-laminitis lameness cases in order to evaluate the capabilities of clinical
83 signs to differentially diagnose laminitis from other causes of lameness. The study is presented considering
84 recommendations
85 of the Strengthening the Reporting of Observational studies in Epidemiology
86 (STROBE) statement (von Elm and others 2007).

87

88 **Materials and Methods**

89 Data were collected from two groups:

90 *Group A*

91 A convenience sample of five veterinary institutions (two referral centres, two large first-opinion and referral
92 equine hospitals and a first-opinion mixed practice) were visited and invited to provide data for this study. In
93 addition, veterinary practices (n=93) that were interested in participating in a parallel epidemiological
94 investigation of equine laminitis, were contacted by telephone or email and invited to provide data on clinical
95 signs of lameness (of any origin) for the study reported here.

96 A literature review was conducted to identify previously suggested clinical signs of laminitis and differential
97 diagnoses. The resultant list was reviewed by expert equine clinicians in selected referral hospitals and
98 laminitis researchers, and a 'lameness reporting form' (LM) (Supplementary Information Item 1) was
99 designed to gather information on laminitis-relevant clinical signs from both laminitic (cases) and non-
100 laminitic lame (controls) horses.

101 Part one of the LM gathered case identifying information with five subsequent sections recording whether
102 clinical signs pertaining to the foot, stance and lameness irregularities (clinical signs) were present, absent or
103 had not been assessed. Part two of the LM allowed practitioners to record their diagnosis as free text and to
104 select specific diagnostic techniques used to confirm the diagnosis from six tick-box options. A free-text
105 comments section was also included for any additional information pertinent to confirmation of the diagnosis.

106 Participating practitioners were asked to complete a LM for equine lameness of any origin seen between
107 February-April 2009, and January 2010-May 2011, with the second phase of data collection initiated to
108 increase numbers for analysis. Completed forms were returned by post using supplied reply-paid envelopes.
109 Upon arrival LMs were divided into two groups for analysis: one group containing reported laminitis cases
110 and another containing all animals for which the primary cause of lameness was not laminitis (controls).

111 *Group B*

112 Following this development phase, a 'laminitis reporting form' (LRF) was finalised (Supplementary
113 Information Item 2) as previously described (Wylie and others 2013a). As for the LM, the LRF consisted of
114 five distinct sections on lameness, stance characteristics, feet affected and observed laminitis-related acute
115 and chronic clinical signs. Based on the data collected from animals in Group A, some modifications to the
116 form were made, hence for the purposes of this study only those clinical signs which were reported for both
117 groups were compared. No further clinical data were recorded for the purposes of this study.

118

119 A LRF was completed for any case of laminitis, defined as a horse or pony with veterinary-diagnosed,
120 clinically apparent laminitis (i.e. an active episode of laminitis), attended by one of the participating
121 practitioners (Wylie and others 2013a). In animals with recurring laminitis, an episode of veterinary-

122 diagnosed active laminitis was defined as new if the animal had returned to its previous/normal level of
123 soundness and had not received analgesic medication for 14 days or more between episodes (Wylie and
124 others 2013a). However, for the purposes of this study only the first episode of laminitis was included.
125 Practices were asked to complete the LRF for all eligible cases occurring from May 2009 to April 2011.

126 *Statistical analysis*

127 To increase the numbers for data analysis, Groups A and B were combined. Multiple different clinical signs
128 were categorised (present, not present or not assessed) under the following five sections:

- 129 (1) Lameness: recumbency, refusal to move unless forced, reluctance to walk, lame at walk, lame at trot,
130 short stilted gait at walk, short stilted gait at trot, difficulty turning
- 131 (2) Stance: shifting weight, front feet placed in front of body, reluctance to lift foot
- 132 (3) Feet affected: bilateral front feet, bilateral hind feet or all four feet
- 133 (4) Acute clinical signs: increased digital pulse, increased hoof temperature, pain on sole pressure
- 134 (5) Chronic clinical signs: Coronary band swelling, coronary band depression, divergent growth rings,
135 change in hoof wall angle, wall separation, flat/convex sole, widened white line, pink crescent dorsal
136 to frog, sole prolapse

137 Initial examination, coding of data and descriptive analyses were conducted using Microsoft Excel (Excel
138 2003, Microsoft). The prevalence (including corresponding 95% confidence intervals [CI]) of each clinical
139 sign, excluding records where the sign was not assessed, in both case and control animals and the between-
140 group differences in prevalence of presence of clinical sign were determined. Associations between each
141 clinical sign and case or control status were tested using logistic regression models reporting adjusted odds
142 ratios (OR) taking into account veterinary practice as a fixed effect, with 95% confidence intervals (CI), and
143 Wald test P-values. All analyses were conducted in R Statistical Package (version 3.1.2 © 2014 The R
144 Foundation for Statistical Computing) using the 'epicalc' and 'tree' packages. Statistical significance was set
145 at a value of $P < 0.05$.

146 Multivariable analysis was carried out using a multi-factorial classification - tree-based statistical models
147 (hereafter ‘tree models’) (Clark and Pregibon 1997). This analytical technique was chosen due to the
148 unbalanced dataset with potentially different combinations of factors present in different horses. The analysis
149 consisted of determining a binary division of the clinical signs prevalence data (laminitis vs. non-laminitis
150 lameness), such that there is the largest difference in terms of prevalence of laminitis vs. non-laminitis
151 lameness for those two subsets of data. One subset of animals with a specific clinical sign is first considered
152 (e.g. those with ‘bilateral forelimb lameness’) and the binary division in terms of any of the other clinical
153 signs resulting in the largest difference in prevalence of laminitis is determined. The other subset is then
154 considered (e.g. those with no ‘bilateral forelimb lameness’) and again the clinical signs for which binary
155 division gives the largest difference in prevalence of laminitis vs. non-laminitis lameness is determined. The
156 different “branches” of the tree are independent of each other in terms of what binary partitions are presented.
157 This binary partitioning is continued for smaller and smaller subsets of data until no differentiation in terms
158 of prevalence is possible. The trees are then ‘pruned’ to exclude very small differentiations based on a few
159 horses. The analysis is presented in graphical form allowing easy comprehension of the grouping of clinical
160 signs giving the largest differences in prevalence in the data. Univariable comparisons of the distribution of
161 clinical signs for particular subsets identified in the trees were then carried out as per the association between
162 clinical signs and case/controls status described above.

163 Five separate preliminary tree models were produced for the following characteristics to represent the
164 features of clinically active laminitis recorded: i) lameness, ii) stance, iii) feet affected, iv) acute signs only
165 and iv) acute and chronic signs. ‘Lame at trot’ and ‘short stilted gait at trot’ were excluded from the lameness
166 tree model due to large numbers of missing data where these signs had not been assessed (missing for 55.0%
167 and 49.4% of observations, respectively).

168 After consideration of the five preliminary trees, those variables identified in each preliminary tree as being
169 the greatest differentiators in terms of laminitis were analysed together to form two combined tree models: (i)
170 a combined model of lameness, stance characteristics, feet affected and observed laminitis-related acute
171 clinical signs to reflect active episodes of laminitis in horses with no evidence of chronic laminitis, and (ii) a
172 combined model of lameness, stance characteristics, feet affected and observed laminitis-related acute and

173 chronic clinical signs to reflect active episodes of laminitis in horses with evidence of previous SADP failure
174 (chronic laminitis).

175

176 **Results**

177 *Recruitment*

178 ***Group A***

179 All five veterinary establishments visited agreed to provide data for this study. In addition, 25 first-opinion
180 veterinary practices agreed to participate, of which 14 (46.7%) contributed data to the study. Lameness forms
181 were provided for 238 unique horses/ponies: 89 (37.4%) from referral practices and 149 (62.6%) from first-
182 opinion practices. Thirty-seven animals (15.5%) were diagnosed by veterinary practitioners as laminitis cases
183 and 201 (84.5%) were diagnosed with non-laminitis lameness. Other causes of lameness included, but were
184 not restricted to, proximal suspensory desmitis (n=40, 17.3%), foot abscesses (n=22, 9.5%) and fractures
185 (n=16, 6.9%). Overall, 73 (30.7%: CI 24.8, 36.5) Group A animals were diagnosed on the basis of clinical
186 signs without further diagnostic procedures (cases 32.4%: CI 17.3, 47.5, controls 30.3%: CI 24.0, 36.7) and
187 155 (65.1%: CI 59.1, 71.2) animals were diagnosed using multiple diagnostic modalities (cases 62.2%: CI
188 46.5, 77.8, controls 65.7%: CI 59.1, 72.2). Stated diagnostic techniques used to investigate lameness in the
189 laminitic cases included clinical examination (94.6%: CI 87.3, 100), radiography (64.9%: CI 49.5, 80.2),
190 regional anaesthesia (nerve blocks) (13.5%: CI 2.5, 24.5), surgical/post-mortem findings (13.5%: CI 2.5,
191 24.5) and blood testing for concurrent predisposing metabolic conditions (8.1%: CI 0.01, 16.9).

192 ***Group B***

193 The recruitment of cases is described in detail in Wylie et al. (2013a). In brief, LRFs were received for 551
194 unique horses/ponies from 30 first-opinion veterinary practices over the two-year period.

195 *Clinical signs*

196 The prevalence of the presence of each clinical sign in laminitis cases and non-laminitis lame controls,
 197 excluding records where the sign was not assessed, and difference in prevalence between the two groups are
 198 provided in Table 1. The overall prevalence of specific clinical signs ranged from 2.7% (CI 1.5, 3.9) for ‘sole
 199 prolapse’ (number assessed = 706) to 85.0% (CI 81.4, 88.7) for ‘lame at trot’ (number assessed = 367). The
 200 difference in prevalence between cases and controls ranged from -14.1% for ‘lame at trot’ (sign more
 201 common in controls) to +71.9% for ‘short stilted gait at walk’ (found more often in cases than controls).
 202 There were five clinical signs with a difference in prevalence of greater than +50%: three lameness-related
 203 signs (‘reluctance to walk’, ‘short, stilted gait at walk’ and ‘difficulty turning’), one stance-related sign
 204 (‘shifting weight’) and one acute clinical sign (‘increased digital pulse’).

205 The logistic regression results are provided in Table 2. For each clinical sign there was a statistically
 206 significant increase in the odds of occurrence in the laminitis (cases) group, with the exception of
 207 ‘recumbent’, ‘lame at trot’ and ‘coronary band swelling’ for which there was no significant difference
 208 ($P>0.05$). No odds ratio could be calculated for ‘coronary band depression’ or ‘sole prolapse’ because no
 209 animals in the control group showed these clinical signs.

210 The preliminary tree models are provided in Supplementary Information Item 3. Consideration of the
 211 lameness tree identified the best discriminator as ‘short stilted gait at walk’; 93.1% (CI 90.6, 95.5) of animals
 212 with that clinical sign had laminitis; 94.1% (CI 91.6, 96.5) of animals with both ‘short stilted gait at walk’
 213 and ‘difficulty turning’ had laminitis. Of the 219 animals that did not have a ‘short stilted gait at walk’, only
 214 27.9% (CI 21.9, 33.8) had laminitis – however, if they had ‘difficulty turning’ 59.7% (CI 48.0, 71.5) had
 215 laminitis. For animals where both these clinical signs were absent, if they were ‘reluctant to walk’ 40.0% (CI
 216 15.2, 64.8) had laminitis.

217 The best discriminator in the stance tree was ‘shifting weight’; 98.1% (CI 96.6, 99.6) of animals with that
 218 clinical sign had laminitis. In animals that were not ‘shifting weight’, ‘front feet placed in front of the body’
 219 identified 94.2% (CI 89.2, 99.1) as laminitis cases.

220 In the 'acute clinical signs' tree, 91.0% (CI 88.5, 93.5) of animals with 'increased digital pulses' had
221 laminitis, and 'pain on sole pressure' in the absence of 'increased digital pulses' identified 69.0% (CI 52.1,
222 85.8) as cases of laminitis.

223 The best discriminator in the 'acute and chronic clinical signs' tree was 'increased digital pulses'; 91.0% (CI
224 88.4, 93.5) of animals with that clinical sign had laminitis, and the additional presence of 'divergent growth
225 rings' identified 100% as laminitis cases.

226 The tree diagram combining categories of clinical signs for acute laminitis with lameness, stance and feet is
227 provided in Figure 1. Presence of 'lameness in both forelimbs' was the best discriminator, with 93.1% (CI
228 90.7, 95.5) of animals with this clinical sign belonging to the laminitis group. Additional presence of an
229 'increased digital pulse' improved diagnostic accuracy to 99% (CI 97.9, 100) ($P<0.001$). A 'bilateral
230 forelimb lameness' with no 'increase in digital pulse', yet presence of a 'short stilted gait at walk' identified
231 100% of animals as laminitis cases, however statistical analysis of this sub-group and the presence of
232 'shifting weight' was not possible due to small numbers of animals with these signs. The presence of 'pain
233 on sole pressure' was not statistically associated with improved clinical discrimination ($P=0.30$).

234 The overall tree diagram considering both acute and chronic laminitis clinical signs with lameness, stance and
235 feet is provided in Figure 2. Presence of 'lameness in both forelimbs' was again the best discriminator; 92%
236 of animals with this clinical sign had laminitis ($P<0.001$). The additional presence of 'increased digital
237 pulses' improved this to 99% of cases ($P<0.001$). Presence of a 'flat/convex sole' also provided improved
238 clinical discrimination ($P=0.002$). It was not possible to assess statistical significance for 'short stilted gait at
239 walk', or 'shifting weight', again because of the small numbers of animals with these signs.

240

241 **Discussion**

242 This is the first study comparing the prevalence of veterinary-recognised clinical signs in laminitis and other
243 causes of lameness to evaluate the capabilities of discrimination for differential diagnosis.

244 A wide range of clinical signs were displayed by the laminitic cases, in agreement with previous reviews
245 (Baxter 1994; Eustace 2010; Hunt and Wharton 2010; Swanson 1999). There were no individual, or
246 combinations of, clinical signs present in every case. The clinical signs that were considered to be the most
247 useful on the basis of this work were three features of lameness investigation ('reluctance to walk', 'short,
248 stilted gait at walk' and 'difficulty turning'), one feature of stance ('shifting weight') and an 'increased digital
249 pulse'. All these signs had a difference in prevalence of over 50% between active laminitis cases (signs more
250 prevalent) and non-laminitic lame horses (signs less prevalent). As the clinical details forms were designed to
251 gather information on laminitis, it may be expected there was a statistically significant difference in the
252 distribution of many of the clinical signs between laminitis cases and non-laminitis lameness controls. For
253 the purposes of this study it was considered important to focus only on the lameness-associated clinical signs
254 for two main reasons. Firstly, because regardless of the underlying pathological process of laminitis, the
255 common feature of all cases of laminitis is the induction of pathological changes within the SADP, resulting
256 in overt foot pain and clinical signs related to lameness (Baxter 1994; Budras and others 2009a; Budras and
257 others 2009b; Eades 2010), and as a consequence previous epidemiological studies of laminitis have used
258 only lameness-associated clinical signs as their case inclusion/exclusion criteria (Alford and others 2001;
259 Dorn and others 1975; Hood and others 1994; Menzies-Gow and others 2010a; Parsons and others 2007;
260 Slater and others 1995). Secondly, to keep the amount of work required by the veterinary surgeons to a
261 minimum to enhance compliance. Collection of data regarding systemic clinical signs would have increased
262 the amount of work required by the participating veterinary practitioners, and it was considered that their
263 presence would aid the diagnosis of the underlying, predisposing condition rather than laminitis directly.
264 Nevertheless, it is acknowledged that as part of the diagnostic process veterinarians will use the animal's
265 history and other clinical features in making their diagnosis. As such, collection of additional clinical data in
266 future studies would be useful to improve the current decision trees, as well as to generate further trees
267 pertaining to, for example, signs of colic.

268 Currently, visual assessment of lameness is a highly subjective process. Many kinetic and kinematic methods
269 for objectively assessing lameness have been reviewed previously (Hood and others 2001; Keegan 2010), and
270 it is possible that these may prove to be more reliable than visual assessment alone in the future (Dyson

271 2011). Further evaluation of techniques to evaluate stance and gait characteristics of lame animals may result
272 in a more objective method of diagnosing and/or scoring laminitis, as well as other reasons for lameness.
273 Recently developed techniques allow assessment of horse movement without impeding the use of the animal,
274 and may have a role in evidence-based assessment of lameness in horses in veterinary practice in the future
275 (Dyson 2011; Keegan 2010; Pfau and others 2007). There was no statistically significant difference in
276 prevalence of 'lameness at trot' between cases and controls, and this variable was not included in the tree
277 analysis due to large number of laminitic cases that were not assessed at trot. The high level of missing data
278 is likely to reflect the appropriate reluctance of veterinary surgeons to trot suspect laminitis cases on welfare
279 grounds and so as not to exacerbate lamellar pathology, and the common use of intrasynovial anaesthesia for
280 diagnosis of other lamenesses commonly evaluated at the trot.

281 Two clinical signs – 'coronary band depression' and 'prolapsed sole' - were pathognomonic for laminitis in
282 this study, . were only found in 13.6% and 3.7% of cases, respectively. Both these signs can indicate disease
283 progression to chronic phase laminitis (i.e. SADP failure and distal phalanx dislocation within the hoof);
284 therefore these signs would not be expected to be present in acute cases, unless they were also suffering from
285 concurrent pathology such as chronic seedy toe/white line disease or severe club feet (Kuwano and others
286 1999). These results may help veterinary practitioners prioritise where to begin their clinical examination of
287 an active laminitis case, as primary inspection of the sole and coronary band would prevent the animal
288 undergoing lameness evaluation which could precipitate further SADP damage/failure.

289 Two overall combined trees were generated to reflect the two clinical scenarios of active laminitis, one
290 consisting of clinical signs considered to occur in the acute phase of the disease, and one that also contained
291 data reflective of lamellar damage and displacement of the SADP. In both scenarios, the presence of a
292 bilateral lameness was the most useful discriminator, followed by the presence of increased digital pulses.
293 Whilst these clinical presentations are not specific for laminitis, this work provides an evidence-base for case
294 diagnosis and future epidemiological case definitions.

295 This work did not provide evidence for some commonly cited clinical signs of diagnostic importance. In
296 particular, 'front feet in front of the body', taken to represent the classic 'laminitis stance', was found in less
297 than half of the diagnosed active laminitis cases, and did not prove to be a useful discriminator. Therefore,

298 despite much anecdotal publicity of this visibly apparent clinical sign (Stashak 2002; Swanson 1999),
299 veterinarians, researchers and owners should be careful to avoid relying on its presence for making a
300 diagnosis of laminitis [40].

301 The use of clinical recording forms based on evidence-based recommendations may help veterinary
302 practitioners structure their clinical examination of an active laminitis case. However, in medical practice
303 well-validated diagnostic algorithms tools are underused (Pearson and others 1994). For example, a simple
304 predictor based on seven clinical signs for ischaemia in humans was only used in 2.8% of cases (Corey and
305 Merenstein 1987). The clinical usefulness of developing such a technique would need to be established by a
306 survey of first-opinion practitioners to decide whether such a tool would provide useful assistance in laminitis
307 diagnosis in the field.

308 The limitations of this study include diagnosis by a number of different veterinary clinicians, which may have
309 different levels of experience. To take this into account veterinary practice was included in the generation of
310 the odds ratio estimates, however, misclassification bias may still occur, although this would have tended to
311 shift the odds ratios towards non-significant. Similarly, as it is not possible to obtain a definitive diagnosis of
312 active laminitis in an observational epidemiological study there was the potential for misclassification of
313 cases and controls. For this reason, veterinary recordings of the clinical signs observed was used, as
314 described in Wylie et al., (Wylie and others 2013a, b) and misclassification would have again reduced the
315 ability to detect significant differences rather than produce anomalous significant differences. Inclusion of
316 data in the tree models required the animals to have data for each included variable, resulting in smaller
317 numbers of contributing individuals as the trees became more complex. Consequently, although the variables
318 retained high statistical significance, smaller contributing sample sizes led to larger confidence intervals
319 around prevalence point estimates and the need therefore for some caution in their interpretation.

320 It is acknowledged that there may be some bias in the data if veterinary practitioners did not accurately detail
321 the clinical signs which they observed and perhaps listed clinical signs that they anticipated to reflect their
322 diagnosis. Furthermore, it would be interesting to collect greater numbers of control animals to conduct the
323 analyses between specific control lamenesses, such as forelimb foot pain only, to highlight more subtle
324 differences between presenting pathologies.

325 In conclusion, separate clinical signs were compared between laminitis and non-laminitis cases of lameness,
326 and no individual sign was present in every case of laminitis. The clinical signs which best indicated a case
327 of laminitis were characteristic of the chronic phase of the disease only. Improved evaluation of the clinical
328 signs displayed by laminitic animals examined by first-opinion practitioners will lead to equine welfare
329 improvements, as the best recoveries occur in animals undergoing intensive treatment within several hours of
330 the appearance of the disease (Redden 1986). Future consensus on a basic disease definition may permit
331 future systematic review and meta-analysis of epidemiological investigations collecting similar information in
332 different locations worldwide.

333

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337

338

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Table 1: Prevalence and 95% confidence intervals (CI) for each clinical sign in both laminitis cases and non-laminitis lameness controls, excluding records where the sign was not assessed, and the percentage of horses that were assessed with corresponding difference in prevalence.

Clinical signs		Cases (n=588)					Controls (n=201)					Overall (n=789)		
		Present (n)	Absent (n)	Prevalence (%)	LCI (%)	UCI (%)	Present (n)	Absent (n)	Prevalence (%)	LCI (%)	UCI (%)	Number assessed	Percentage assessed (%)	Difference in prevalence (%)
Lameness	Recumbent	24	479	4.8	2.9	6.6	1	191	0.5	0.0	1.5	695	88.1	+4.3
	Refusal to move unless forced	148	361	29.1	25.1	33.0	14	180	7.2	3.6	10.9	703	89.1	+21.9
	Reluctance walk	395	155	71.8	68.1	75.6	38	157	19.5	13.9	25.1	745	94.4	+52.3
	Lame walk	409	95	81.2	77.7	84.6	76	122	38.4	31.6	45.2	702	89.0	+42.8
	Lame trot	152	42	78.4	72.6	84.2	160	13	92.5	88.6	96.4	367	46.5	-14.1
	Short stilted walk	446	66	87.1	84.2	90.0	29	162	15.2	10.1	20.3	703	89.1	+71.9
	Short stilted trot	125	55	69.4	62.7	76.2	53	119	30.8	23.9	37.7	352	44.6	+38.6
	Difficulty turning	456	47	90.7	88.1	93.2	52	137	27.5	21.2	33.9	692	87.7	+63.1
Stance	Shifting weight	316	256	55.2	51.2	59.3	7	188	3.6	1.0	6.2	767	97.2	+51.7
	Front feet in front	250	317	44.1	40.0	48.2	6	190	3.1	0.7	5.5	763	96.7	+41.0
	Reluctance lift foot	300	269	52.7	48.6	56.8	24	169	12.4	7.8	17.1	762	96.6	+40.3
Feet Affected	Bilateral fore	538	44	92.4	90.3	94.6	32	152	17.4	11.9	22.9	766	97.1	+71.7
	Bilateral hind	244	323	43.0	39.0	47.1	25	156	13.8	8.8	18.8	748	94.8	+28.3
	All four feet	234	348	40.2	36.2	44.2	5	193	2.5	0.3	4.7	780	98.9	+39.5
Acute	Increased digital pulse	520	50	91.2	88.9	93.6	45	150	23.1	17.2	29.0	765	97.0	+68.2
	Increased hoof temperature	324	218	59.8	55.7	63.9	30	164	15.5	10.4	20.6	736	93.3	+44.3
	Pain sole pressure	263	271	49.3	45.0	53.5	35	149	19.0	13.4	24.7	718	91.0	+30.2
Chronic	Coronary band swelling	27	505	5.1	3.2	6.9	6	186	3.1	0.7	5.6	724	91.8	+2.0
	Coronary band depression	73	462	13.6	10.7	16.6	0	192	0.0	0.0	0.0	727	92.1	+13.6
	Divergent growth rings	148	378	28.1	24.3	32.0	3	190	1.6	0.0	3.3	719	91.1	+26.6

Change hoof wall angle	129	383	25.2	21.4	29.0	7	186	3.6	1.0	6.3	705	89.4	+21.6
Wall separation	71	445	13.8	10.8	16.7	2	184	1.1	0.0	2.6	702	89.0	+12.7
Flat/convex sole	232	291	44.4	40.1	48.6	9	180	4.8	1.7	7.8	712	90.2	+39.6
Widened white line	133	368	26.6	22.7	30.4	8	176	4.4	1.4	7.3	685	86.8	+22.2
Pink crescent	46	464	9.0	6.5	11.5	1	189	0.5	0.0	1.6	700	88.7	+8.5
Sole prolapse	19	498	3.7	2.1	5.3	0	189	0.0	0.0	0.0	706	89.5	+3.7

429 Table 2: Odds ratios and 95% confidence intervals (CI), with corresponding Wald *P*-values,
 430 for each clinical sign in laminitis cases compared to non-laminitis lameness controls. ORs are
 431 adjusted for the effect of veterinary practice.

432

Clinical Signs		Number	Adjusted Odds Ratio	95% Confidence Interval	Wald P-value
Lameness	Recumbent	695	5.1	0.5, 51.4	0.17
	Refusal to move unless forced	703	3.5	1.6, 7.7	0.002
	Reluctance walk	745	4.4	2.2, 8.6	<0.001
	Lame walk	702	2.2	1.0, 4.7	0.04
	Lame trot	367	0.3	0.0, 2.6	0.29
	Short stilted walk	703	9.4	4.5, 19.6	<0.001
	Short stilted trot	352	3.9	1.6, 9.6	0.003
	Difficulty turning	692	16.9	7.0, 40.8	<0.001
Stance	Shifting weight	767	17.7	6.8, 45.6	<0.001
	Front feet in front	763	24.5	7.9, 75.9	<0.001
	Reluctance lift foot	762	4.0	1.9, 8.1	<0.001
Feet Affected	Bilateral fore	766	40.5	16.3, 100.9	<0.001
	Bilateral hind	748	21.3	7.7, 59.1	<0.001
	All four feet	780	96.3	22.1, 419.8	<0.001
Acute	Increased digital pulse	765	13.2	6.0, 29.3	<0.001
	Increased hoof temperature	736	5.7	2.8, 11.5	<0.001
	Pain sole pressure	718	2.7	1.4, 5.3	0.005
Chronic	Coronary band swelling	727	1.1	0.3, 3.9	0.88
	Coronary band depression	724	NA	NA	NA
	Divergent growth rings	719	96.3	17.1, 542.8	<0.001
	Change hoof wall angle	705	21.1	6.3, 71.0	<0.001
	Wall separation	702	58.5	5.1, 672.8	<0.001
	Flat/convex sole	712	15.5	5.9, 40.5	<0.001
	Widened white line	685	17.3	5.5, 54.5	<0.001
	Pink crescent	700	16.5	2.0, 136.5	0.009
	Sole prolapse	706	NA	NA	NA

433 Figure 1: Tree diagram of the occurrence of laminitis for combinations of lameness, stance,
434 feet affected, and acute laminitis clinical signs. Data were from 586 horses/ponies for which
435 information on each clinical sign was described, of which 74% had laminitis. The percentage
436 at the end of each branch are the occurrence rates of laminitis in those horses/ponies with that
437 particular combination of clinical signs, and the value in brackets the number of
438 horses/ponies of that particular combination of clinical signs.

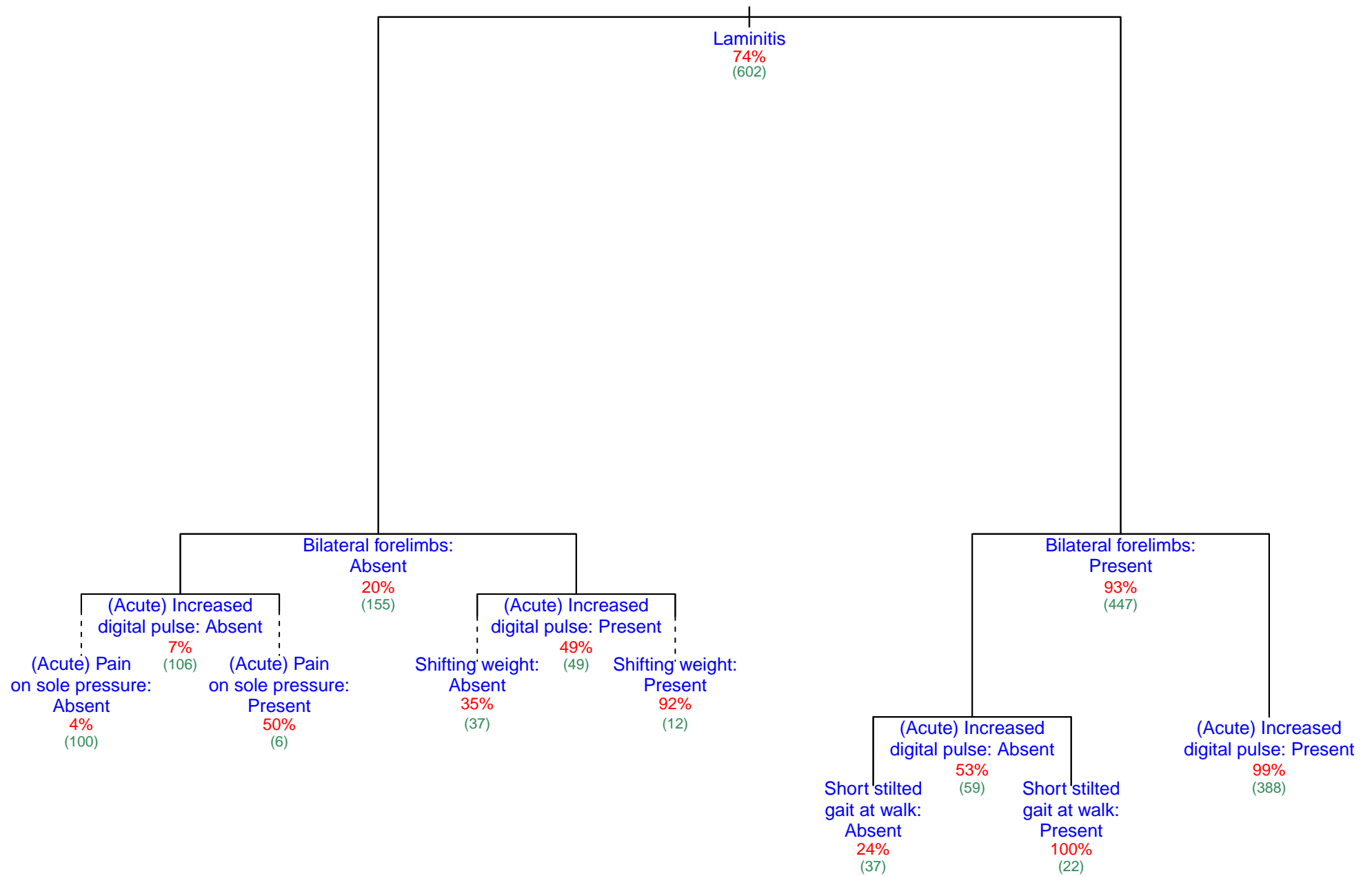
439 Figure 2: Overall tree diagram of the occurrence of laminitis for combinations of lameness,
440 stance, feet affected, acute and chronic laminitis clinical signs. Data were from 551
441 horses/ponies for which information on each clinical sign was described, of which 72% had
442 laminitis. The percentage at the end of each branch are the occurrence rates of laminitis in
443 those horses/ponies with that particular combination of clinical signs, and the value in
444 brackets the number of horses/ponies of that particular combination of clinical signs.

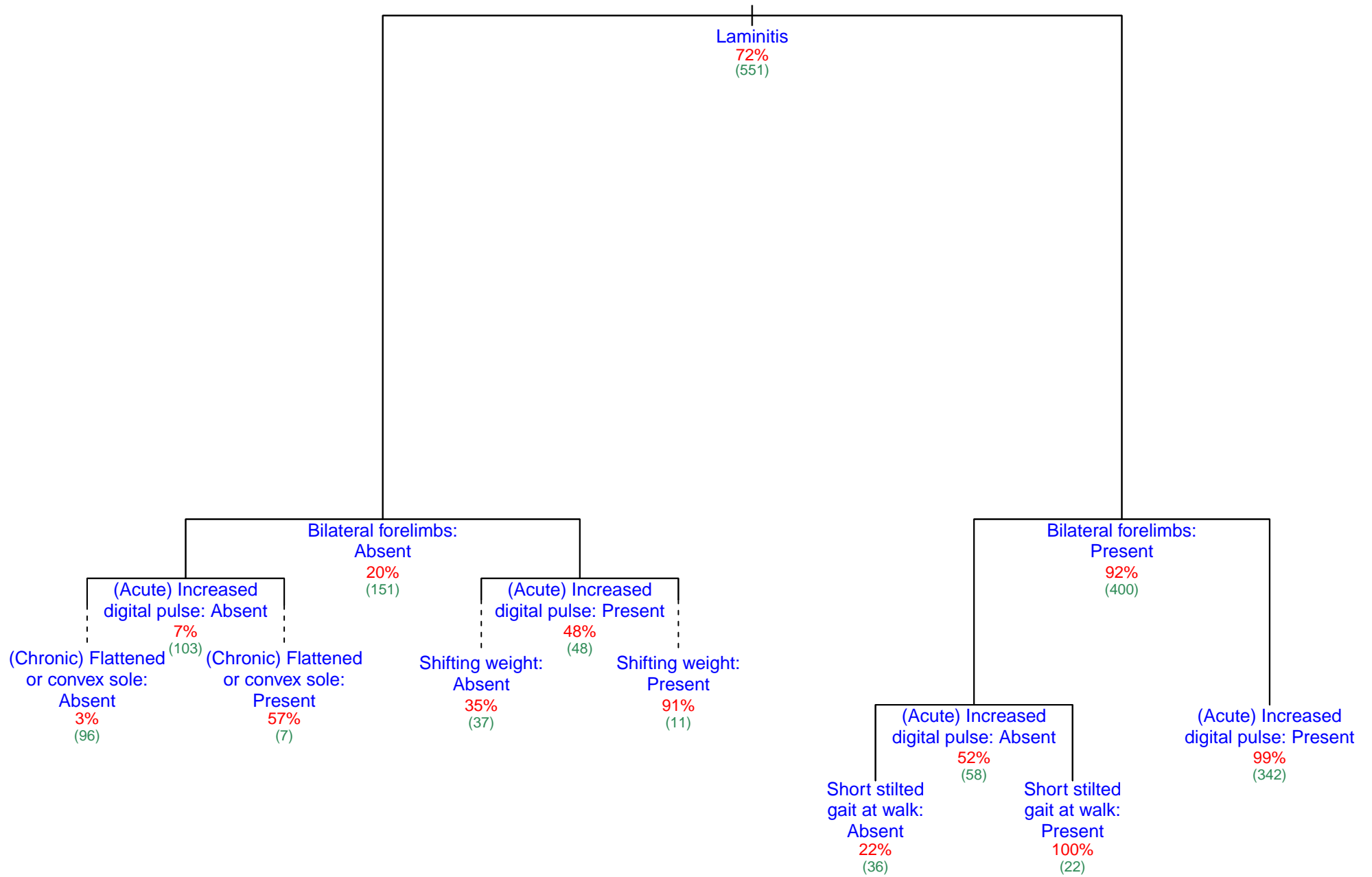
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449 Supplementary Information Item 1: Lameness reporting form (LM) used to investigate the
450 clinical signs of laminitis in Group A recruiting both cases and controls.

451 Supplementary Information Item 2: Laminitis reporting form (LRF) used to investigate the
452 clinical signs of laminitis in Group B recruiting cases only.


453 Supplementary Information Item 3: Preliminary Tree models of the occurrence of laminitis
454 for combinations of lameness, stance, feet affected, acute and chronic laminitis clinical signs.





- 1 Supplementary Information Item 1: Lameness reporting form (LM) used to investigate the
2 clinical signs of laminitis in Group A recruiting both cases and controls.

3

LAMENESS REPORTING FORM 1		 Animal Health Trust	
Name of horse/pony: _____			
Surname of owner/Case I.D.: _____			
Date of clinical examination: <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>			
Lameness	Assessment (please circle 1 option per line)		
Recumbent	Yes / No / Didn't assess		
Refusal to move unless forced	Yes / No / Didn't assess		
Reluctance to walk	Yes / No / Didn't assess		
Lame at walk	Yes / No / Didn't assess		
Lame at trot	Yes / No / Didn't assess		
Short, stilted gait at walk	Yes / No / Didn't assess		
Short, stilted gait at trot	Yes / No / Didn't assess		
Difficulty turning	Yes / No / Didn't assess		
Stance	Assessment (please circle 1 option per line)		
Shifting weight	Yes / No / Didn't assess		
Front feet placed in front of body	Yes / No / Didn't assess		
Front feet placed underneath body	Yes / No / Didn't assess		
Square stance	Yes / No / Didn't assess		
Reluctance for a foot to be lifted	Yes / No / Didn't assess		
Feet affected	Assessment (please circle 1 option per line)	Most severely affected foot/feet (please tick all that apply)	
Right fore	Yes / No / Didn't assess		
Left fore	Yes / No / Didn't assess		
Right hind	Yes / No / Didn't assess		
Left hind	Yes / No / Didn't assess		
Clinical signs of the <u>most</u> severely affected foot/feet	Assessment (please circle 1 option per line)		
Increased digital pulse	Yes / No / Didn't assess		
Increased hoof temperature	Yes / No / Didn't assess		
Decreased hoof temperature	Yes / No / Didn't assess		
Pain on sole pressure	Yes / No / Didn't assess		
Coronary band swelling	Yes / No / Didn't assess		
Coronary band depression	Yes / No / Didn't assess		
Clinical signs of the <u>most</u> severely affected foot/feet	Assessment (please circle 1 option per line)		
Divergent growth rings (wider at heels)	Yes / No / Didn't assess		
Change in dorsal hoof wall angle	Yes / No / Didn't assess		
Wall separation	Yes / No / Didn't assess		
Flattened or convex sole	Yes / No / Didn't assess		
Widened white line	Yes / No / Didn't assess		
Pink crescent dorsal to frog	Yes / No / Didn't assess		
Prolapsed sole	Yes / No / Didn't assess		
For office use only: Animal Health Trust 1361			
AHT Reference: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>			


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6

7 Supplementary Information Item 2: Laminitis reporting form (LRF) used to investigate the
8 clinical signs of laminitis in Group B (cases only).

9



ANIMAL HEALTH TRUST
LAMINITIS REPORTING FORM



WorldHorseWelfare
the new name for the EHR

1. Name of horse/pony:

2. Surname of owner/Case ID:

3. Date of clinical examination: / /

4. Lameness	Assessment (please cross one option per line)		
	YES	NO	DIDN'T ASSESS
Recumbent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Refusal to move unless forced	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Reluctance to walk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lame at walk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lame at trot	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Short, stilted gait at walk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Short, stilted gait at trot	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Difficulty turning	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

5. Stance	Assessment (please cross one option per line)		
	YES	NO	DIDN'T ASSESS
Shifting weight	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Leg trembling	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Front feet placed in front of body	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hind feet placed underneath body	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Reluctance for a foot to be lifted	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

6. Feet affected	Affected (please cross one option per line)			Most severely affected foot (or feet if bilaterally affected) (please cross all that apply)
	YES	NO	DIDN'T ASSESS	
Right fore	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Left fore	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Right hind	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Left hind	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>


7. Clinical signs of the most severely affected foot/feet	Assessment (please cross one option per line)		
	YES	NO	DIDN'T ASSESS
Increased digital pulse	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Increased hoof temperature	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Focal sole pain in front of frog	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Generalised dorsal hoof wall pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Coronary band swelling	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Coronary band depression	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

8. Clinical signs of the most severely affected foot/feet	Assessment (please cross one option per line)		
	YES	NO	DIDN'T ASSESS
Divergent growth rings (wider at heels)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Deviation in dorsal hoof wall angle	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wall separation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Flat sole	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Convex sole	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Abnormally wide white line	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Pink crescent/bruising in front of frog	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Prolapsed sole	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Please remember to complete and return BOTH sides of this form.

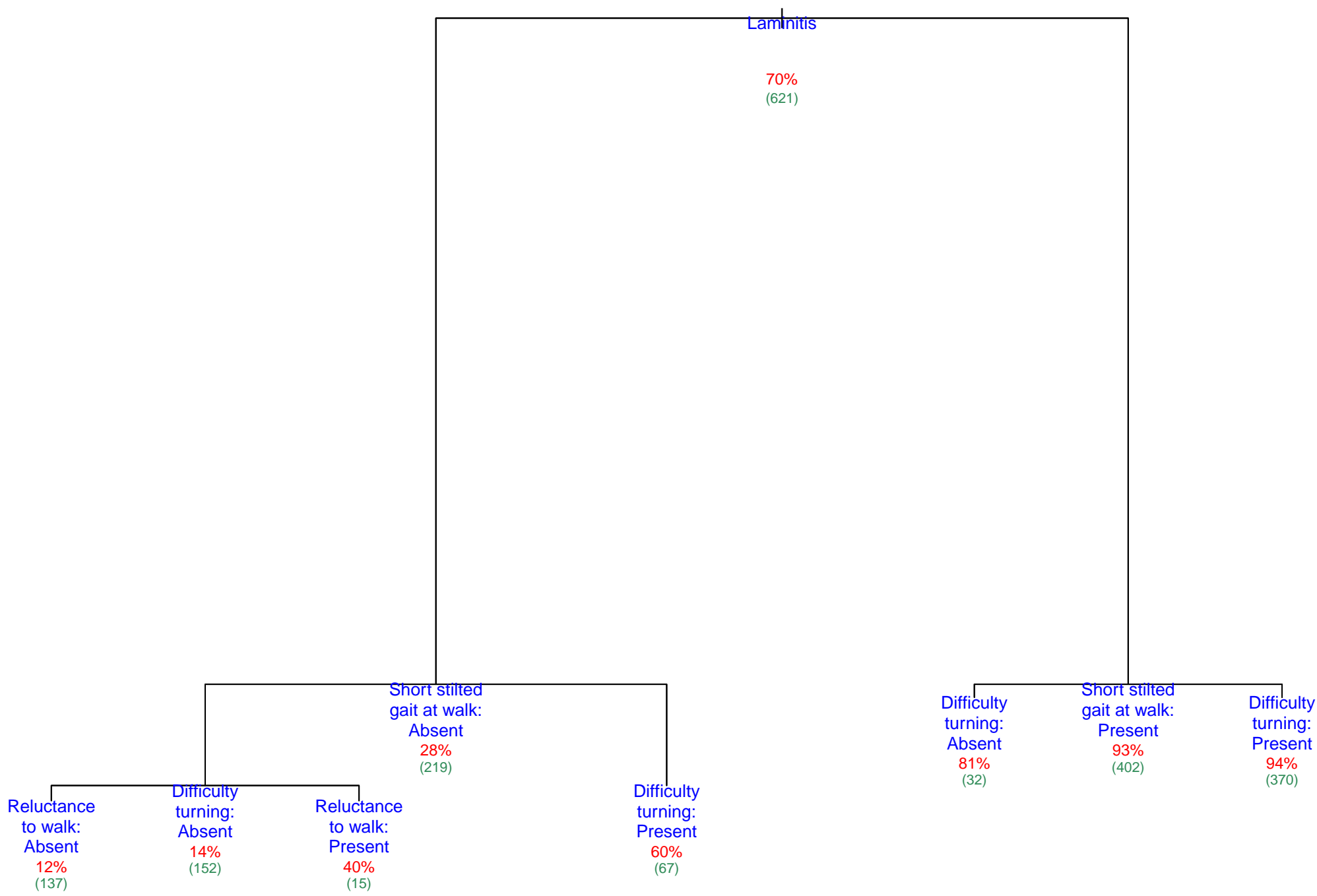
For office use only: P.I.D: Q.I.D: H.I.D:

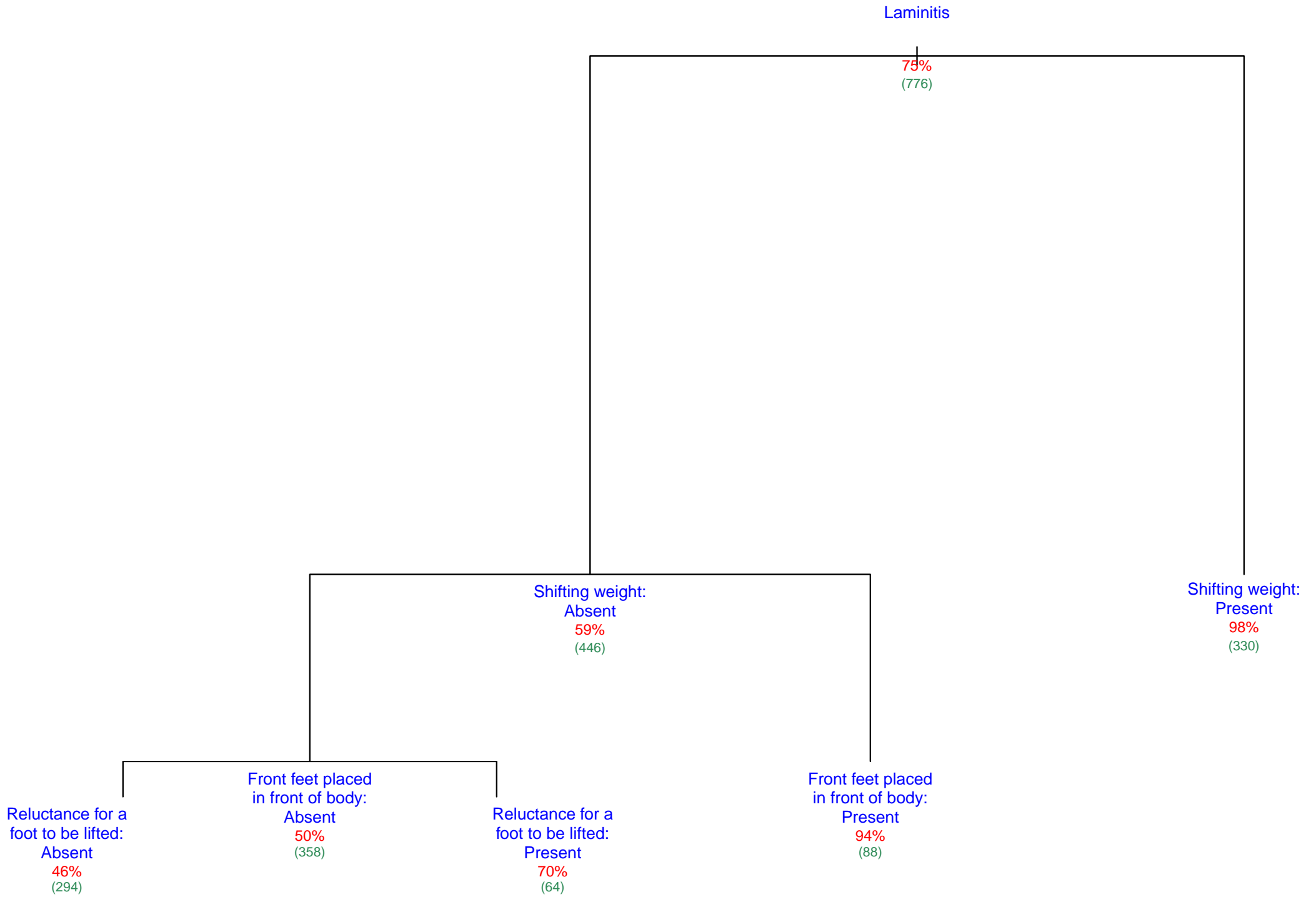
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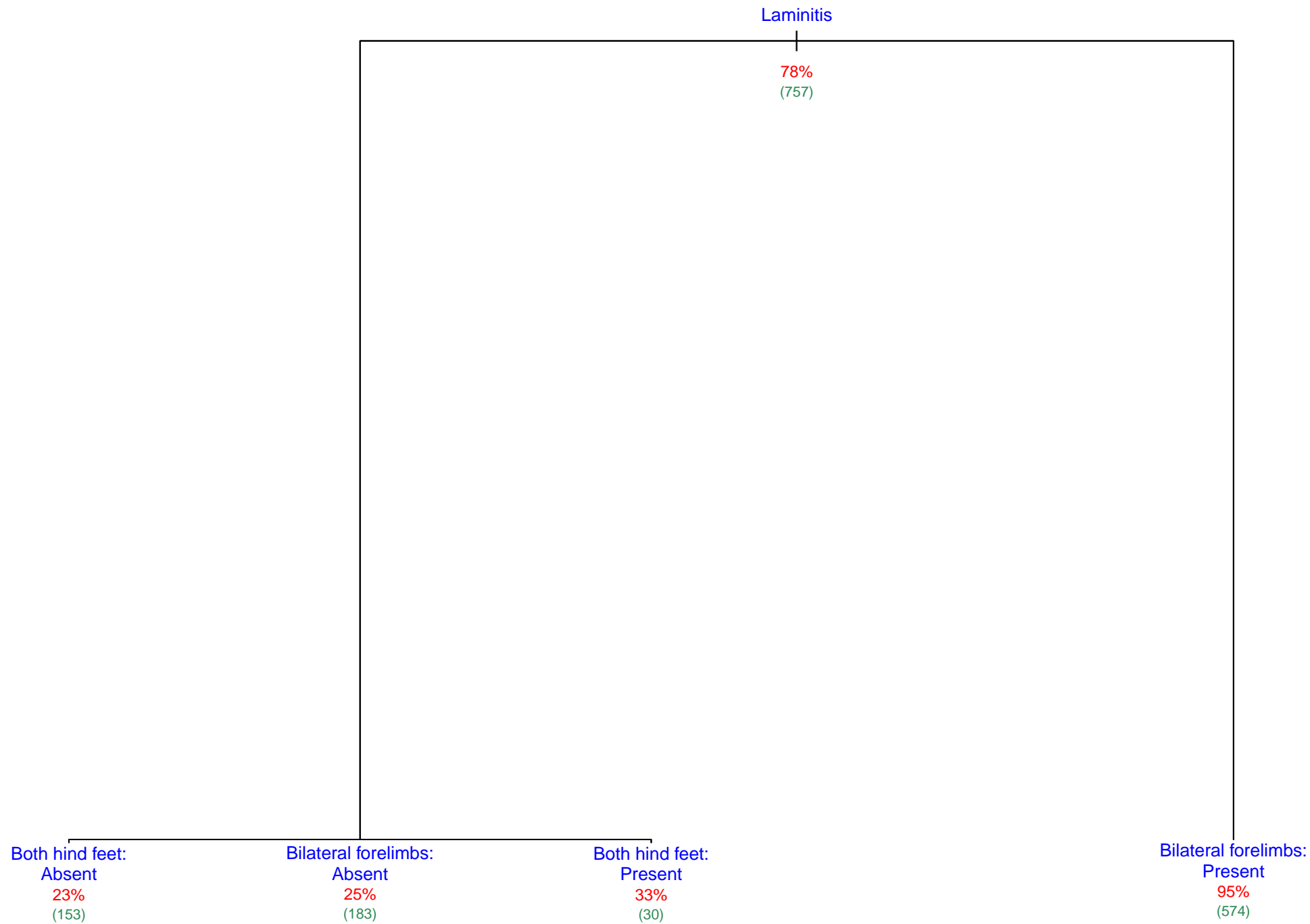


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Laminitis

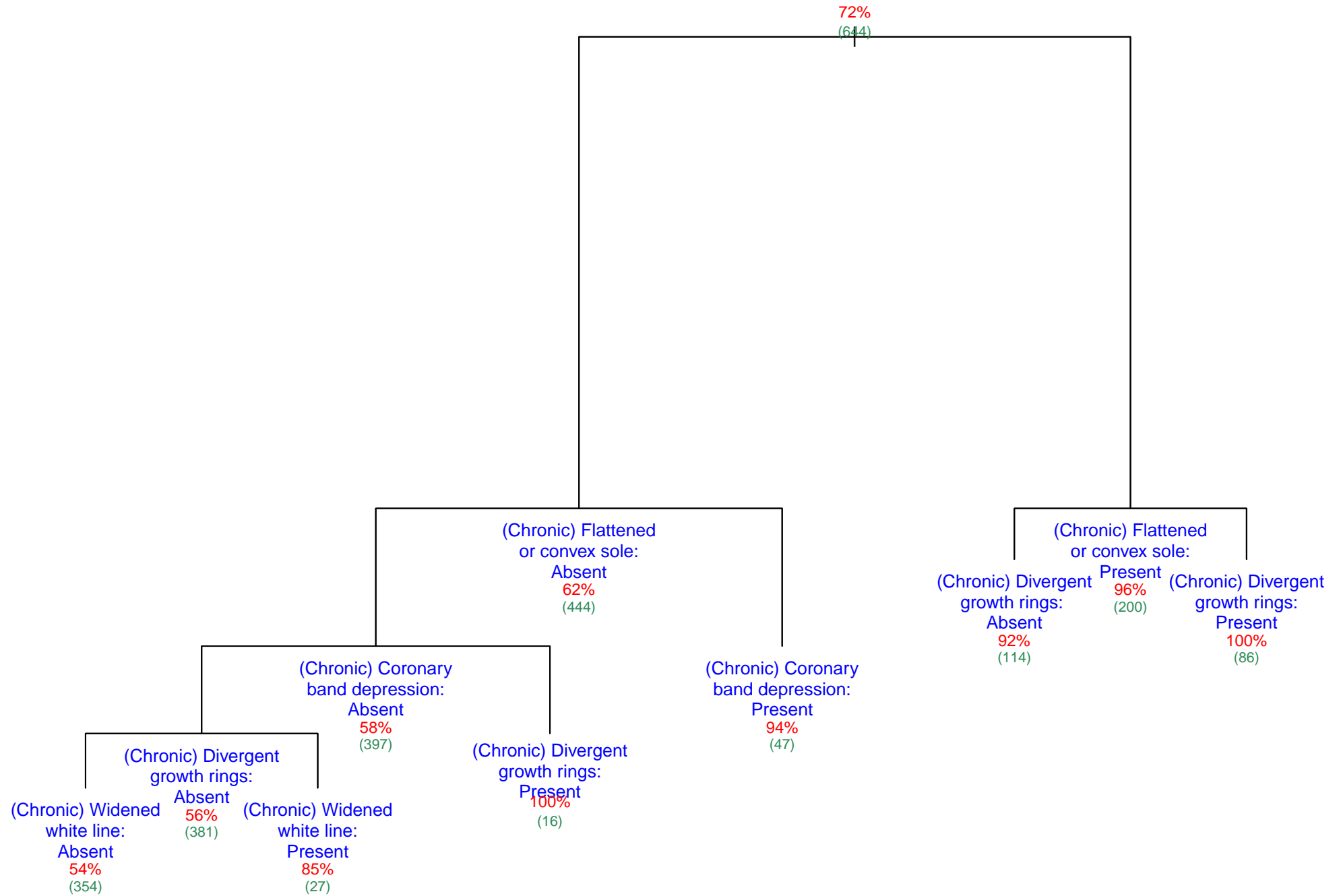
74%
(709)

(Acute) Increased
digital.pulse: Absent
27%
(192)

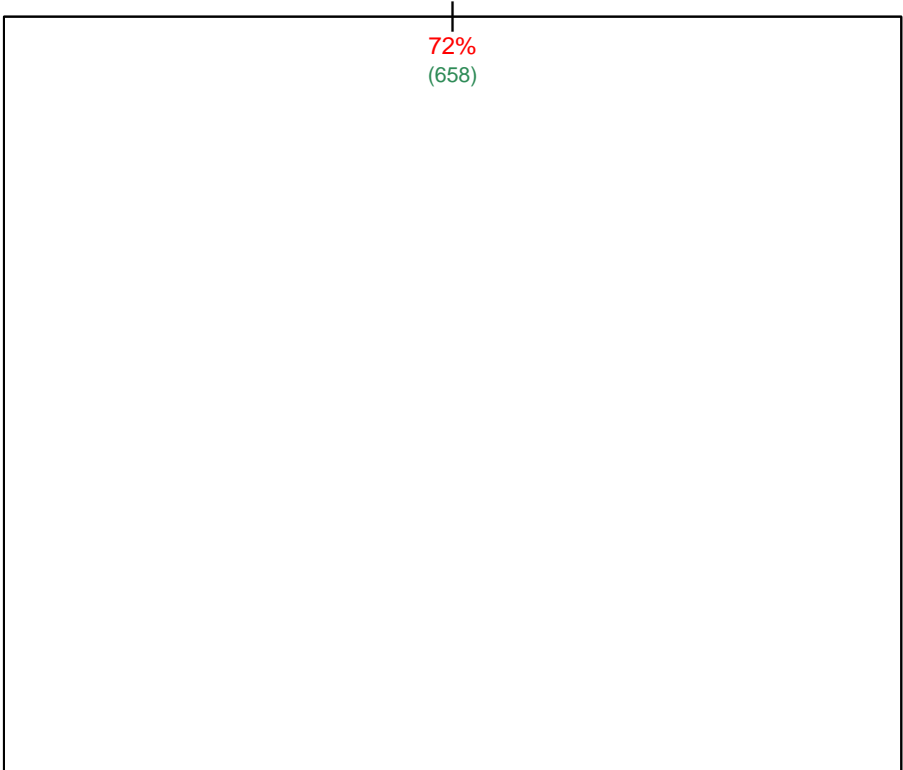
(Acute) Pain
on sole pressure:
Absent
19%
(162)

(Acute) Pain
on sole pressure:
Present
70%
(30)

(Acute) Increased
digital pulse: Present
92%
(517)



Laminitis



(Chronic) Flattened or convex sole:
Absent
62%
(450)

(Chronic) Flattened or convex sole:
Present
96%
(208)

(Chronic) Divergent growth rings:
Absent
58%
(403)

(Chronic) Divergent growth rings:
Present
94%
(47)

(Chronic) Divergent growth rings:
Absent
92%
(115)

(Chronic) Divergent growth rings:
Present
100%
(93)

(Chronic) Widened white line/
Wall separation:
Absent
56%
(372)

(Chronic) Widened white line/
Wall separation:
Present
84%
(31)

(Chronic) Coronary band change:
Absent
91%
(34)

(Chronic) Coronary band change:
Present
100%
(13)

(Chronic) Coronary band change:
Absent
54%
(347)

(Chronic) Coronary band change:
Present
84%
(25)